L4 ANSWER 1 OF 3 MEDLINE
AN 95078777 MEDLINE
DN 95078777 PubMed ID: 7987261
TI Expression cloning, purification and characterization of a beta-1,4-mannanase from Aspergillus aculeatus.
AU Christgau S; Kauppinen S; **Vind J**; Kofod L V; Dalboge H
CS GeneExpress, Novo Nordisk A/S, Copenhagen, Denmark.
SO BIOCHEMISTRY AND MOLECULAR BIOLOGY INTERNATIONAL, (1994 Aug) 33 (5) 917-25.
Journal code: BOD; 9306673. ISSN: 1039-9712.
CY Australia
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-L35487
EM 199501
ED Entered STN: 19950124
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Entered Medline: 19950112

L4 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2001 ACS
AN 2000:608878 CAPLUS
DN 133:188889
TI **Fungal** cells with inactivated DNA mismatch repair system and their use as cloning and expression hosts
IN Borchert, Torben Vedel; Christiansen, Lars; **Vind, Jesper**
PA Novo Nordisk A/S, Den.
SO PCT Int. Appl., 58 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2000050567	A1	20000831	WO 2000-DK63	20000217

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRAI DK 1999-253 A 19990224
RE.CNT 5
RE
(1) Huber, D; Database SWISS-PROT 1998
(2) Maxygen Inc; WO 9831837 A1 1998 CAPLUS
(3) Setratech; WO 9007576 A1 1990 CAPLUS
(4) Setratech; WO 9705268 A1 1997 CAPLUS
(5) Setratech S A R L; WO 9737011 A1 1997 CAPLUS

L4 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2001 ACS
AN 2000:291226 CAPLUS
DN 132:319501
TI Methods of constructing and screening a DNA **library** of interest in filamentous **fungal** cells
IN **Vind, Jesper**
PA Novo Nordisk A/s, Den.
SO PCT Int. Appl., 81 pp.
CODEN: PIXXD2
DT Patent

LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2000024883	A1	20000504	WO 1999-DK552	19991013
	W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	AU 9961885	A1	20000515	AU 1999-61885	19991013
	EP 1124949	A1	20010822	EP 1999-948721	19991013
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
PRAI	DK 1998-1375	A	19981026		
	DK 1999-718	A	19990525		
	WO 1999-DK552	W	19991013		

RE.CNT 4
RE
(1) Aleksenko, A; Fungal Genetics and Biology 1997, V21, P373 CAPLUS
(2) Aleksenko, A; Mol Gen Genet 1996, V253, P242 CAPLUS
(3) Alexei, A; Molecular Microbiology 1996, V20(2), P427
(4) Gems, D; Curr Genet 1993, V24, P520 CAPLUS

=>

B Most **fungi** produce several enzymes simultaneously making the classical enzyme products a mixt. of different enzymes "contaminating" the one enzyme of interest. Although the enzyme mixt. can be used in certain applications, enzymes produced by recombinant technol. are now being introduced on the market. However the time-consuming std. cloning process, based on **enzyme** purifn., amino acid sequence detn. and subsequent probing of **libraries** with DNA probes hampers introduction of cloned products. Recently a new method for fast and efficient isolation of enzyme genes from filamentous **fungi** was described. The method combines the ability of Saccharomyces cerevisiae to **express** heterologous genes with the utilization of sensitive and reliable enzyme assays. A cDNA library is constructed in an S. cerevisiae/E. coli shuttle vector in E. coli from the **fungi** of interest. Plasmid DNA is isolated from library sub-pools and transformed into S. cerevisiae. Next the yeast transformants are replicated onto sets of agar plates contg. appropriate enzyme substrates allowing detection of enzyme activity. After subsequent characterization of clones by DNA sequence anal. a representative cDNA for each enzyme is sub-cloned in an Aspergillus vector and **expressed** in high levels in Aspergillus. More than 200 different enzyme genes encoding enzymes such as arabinanases, endo-glucanases, galactanases, mannanases, polygalacturonases, pectin lyases, pectin Me esterases, proteases, rhamnogalacturonases, lipases and xylanases as well as exo-acting enzymes have been cloned using this new method.

TI Screening and **expression** cloning of **fungal** enzyme genes of industrial relevance

AU Dalboge, Henrik

SO Stud. Org. Chem. (Amsterdam) (1998), 53(New Frontiers in Screening for Microbial Biocatalysts), 29-36
CODEN: SOCHDQ;

The authors have developed a method for fast and efficient isolation of enzyme genes from filamentous **fungi** by combining the ability of Saccharomyces cerevisiae to **express** heterologous genes with the utilization of sensitive and reliable enzyme assays. A cDNA library from the **fungus** Humicola insolens was constructed in a S. cerevisiae/Escherichia coli shuttle vector in E. coli. Sub-pools of the **library** were subsequently screened for **enzyme** activity in S. cerevisiae. More than 130 clones were identified as pos. in either an endo-.beta.-glucanase or an endo-xylanase assay. Based on a partial characterization of the DNA sequence of the individual clones, they could be grouped into five distinct types of endo-.beta.-glucanases and three types of endo-xylanases. A representative cDNA from each type was sub-cloned in an Aspergillus vector and **expressed** in A. oryzae. The new cloning method may be an important alternative to traditional cloning methods based on amino acid sequence information.

TI A novel method for efficient **expression** cloning of **fungal** enzyme genes

AU Dalboege, H.; Heldt-Hansen, H. P.

SO Mol. Gen. Genet. (199

A review with 21 refs. Expression cloning is a relatively new method for fast and efficient cloning of **enzyme** genes from **fungi** that are known to make complex **enzyme** mixts. In contrast to traditional cloning methods that are usually dependent on knowledge of at least a partial amino acid sequence in order to synthesize appropriate DNA probes or **primers**, the expression cloning method solely relies on access to reliable and sensitive **enzyme** assays. A representative expression cDNA **library** is made in Saccharomyces cerevisiae from the donor strain and relevant cDNA **clones** are detected directly based on the encoded **enzyme** activity. Thus, time-consuming **enzyme** purifn. and characterization steps are avoided. The method has been applied on the characterization of extracellular **enzyme** genes from the filamentous **fungus** Aspergillus aculeatus and has resulted in the isolation of 20 different **enzyme** genes such as endo-glucanases, xylanases, pectinases, proteases, hemicellulases and rhamnogalacturonan-degrading **enzymes** . All **enzymes** have been expressed in Aspergillus oryzae, purified and characterized. In the present review a description of the expression cloning technique will be given as well as examples of how the technique has been used in the exploration and characterization of a com. **enzyme** product that is known to consist of a complex mixt. of >25 different **enzyme** activities.

TI Expression cloning of **fungal enzyme** genes; a novel approach for efficient isolation of **enzyme** genes of industrial relevance

AU Dalboge, Henrik

SO FEMS Micr

Expression cloning has been used to isolate a cDNA encoding .beta.-1,4-galactanase from the filamentous **fungus** Aspergillus aculeatus. A cDNA **library** was prepd. from mycelia, inserted in a yeast expression vector and **transformed** into Saccharomyces cerevisiae. Thirteen **clones** secreting galactanase activity were identified from a screening of approx. 2.5 .times. 104 yeast colonies. All **clones** expressed transcripts of the same galactanase gene. The cDNA was re-cloned in an Aspergillus expression vector and **transformed** into Aspergillus oryzae. The recombinant **enzyme** had a mol. wt. of 44 000 Da, an isoelec. point of pH 2.85, a pH optimum of pH 4.0-4.5, and a temp. optimum of 45-65.degree., which is similar to values obtained for a .beta.-1,4-galactanase purified from A. aculeatus. The **enzyme** degraded unsubstituted galactan to galactose and galactobiose. The deduced primary sequence of the **enzyme** showed no apparent homol. to any known **enzyme**, in accordance with this being the first reported .beta.-1,4-galactanase cDNA. However, the deduced amino-acid sequence of a Bacillus circulans DNA sequence contg. an open reading frame (ORF) with no known function, showed 36% identity and 60% similarity to the galactanase amino-acid sequence.

TI Expression cloning, purification and characterization of a .beta.-1,4-galactanase from Aspergillus aculeatus

AU Christgau, Stephan; Sandal, Thomas; Kofod, Lene Venke

SO Curr. Genet. (1995), 27(2), 135-41
CODEN: CUGED5; ISSN: 0172-8083

L32 ANSWER 13 OF 24 CA COPYRIGHT 2000 ACS

AB A cDNA **library** from the filamentous **fungus** A. aculeatus was constructed in the yeast expression vector pYES2.0 and used to isolate 57 full length cDNAs encoding endo-.beta.-1,4-mannanase (I) by expression in S. cerevisiae. The pos. **clones** were identified on agar plates contg. 0.2% azurine-dyed crosslinked mannan by the formation of blue halos around the colonies. All **clones** represented transcripts of the same I gene (man1). The gene was subcloned into an Aspergillus expression vector and **transformed** into A. oryzae for overexpression and purifn. of the **enzyme**. Recombinant I had a mol. wt. of 45 kDa, a pI of 4.5, a pH optimum of pH 5.0, and a temp. optimum of 60-70.degree..

TI Expression cloning, purification and characterization of a .beta.-1,4-mannanase from Aspergillus aculeatus

AU Christgau, Stephan; Kauppinen, Sakari; Vind, Jesper; Kofod, Lene V.; Dalboege, Henrik

SO Biochem. Mol. Biol. Int. (1994), 33(5), 917-25
CODEN: BMBIES

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(71) Applicant: NOVO NORDISK A/S [DK/DK]; Corporate Patents, Novo Alle, DK–2880 Bagsværd (DK).

(72) Inventor: VIND, Jesper; Bagsværdvej 115, DK–2800 Lyngby (DK).

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(54) Title: CONSTRUCTING AND SCREENING A DNA LIBRARY OF INTEREST IN FILAMENTOUS FUNGAL CELLS

(57) Abstract

A method of constructing and screening a library of polynucleotide sequences of interest in filamentous fungal cells by use of an episomal replicating AMA1–based plasmid vector, thus achieving a high frequency of transformation and a stable and standard uniformly high level of gene expression.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 99/00552

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C12N 15/11, C12N 15/80 // C12N 9/00 (C12N 15/80, C12R 1:66)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Curr Genet, Volume 24, 1993, D.H. Gems et al, "Co-transformation with autonomously-replicating helper plasmids facilitates gene cloning from an Aspergillus nidulans gene library", page 520 - page 524, see p. 520, col. 2, l. 7-15; p. 522, col. 2, l. 41 - p. 523, col.1, l. 25; p. 523, col. 2, l. 27-48	1-29
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X	Fungal Genetics and Biology, Volume 21, 1997, A. Aleksenko et al, "Autonomous Plasmid Replication in Aspergillus nidulans: AMA1 and MATE Elements", page 373 - page 387, see p. 383, col. 1, l. 24 - p. 384, col. 1, l. 22	24-27
A		1-23,28-29
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[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 March 2000	10 -03- 2000
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Patrick Andersson/ELY Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 99/00552

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Molecular Microbiology, Volume 20, No 2, 1996, Alexei Aleksenko et al, "Multiple copies of MATE elements support autonomous plasmid replicationin Aspergillus niculans" page 427 - page 434 ---	1-29
A	Mol Gen Genet, Volume 253, 1996, A. Aleksenko et al, "Gene expression from replicating plasmids in Aspergillus nidulans" page 242 - page 246 --- --------	1-29

Form PCT/ISA/210 (continuation of second sheet) (July 1992)